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Technique for Cardiovascular Monitoring in Awake Tethered Rats

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In the rat, reference values for cardiac output vary from 20.7 to 51.8 ml·min^{-1}·100g^{-1} (1,2) and mean arterial pressure from approximately 90 to 175 mm Hg depending on the strain, state of the animal, and method of measurement. Anesthetic agents have been shown to alter the stability of various hemodynamic parameters, as well as, the reactivity of the cardiovascular system to various physiologic and pharmacologic manipulations (3,4,5). Additionally, it has been shown that the stress of physical restraint results in elevated blood pressure in normotensive rats (6) and enhanced response to stress in spontaneously hypertensive rats (7). Therefore, it is important to develop an awake model free from the influence of anesthesia and physical restraint. Although the rat has been used extensively in cardiovascular research, few report use of thermodilution cardiac output in the awake, unrestrained rat (8,9). Likewise, it is rare to find simultaneous and repeated measurement of electrocardiogram (ECG) and arterial blood pressure in awake rats. Therefore, we developed a simple technique to assess cardiac output, ECG, and arterial blood pressure in the awake, freely moving state. Values for these parameters in the conscious F344 rats are reported.

Seventy-six mature (250-300g) F344 derived rats[1] were utilized. The rats were initially maintained four to five per group in metal cages containing autoclaved pine shaving bedding. They were given water *ad libitum*, and commercial rat diet. Ambient temperatures were maintained between 20-23°C and light was on a 12 hour light/dark cycle. Housing and husbandry conditions meet guidelines established in the Guide for the Care and Use of Laboratory Animals. Once surgically instrumented, the rats were housed individually for study as described below. ECG and direct arterial blood pressure were measured in one set of animals (model 1) while cardiac output via the thermodilution technique was measured in a second set of animals (model 2). Anesthesia was induced for surgical preparation with sodium pentobarbital (40 mg/kg i.p.). The animals were prepared for surgery by clip-

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ping the hair followed by surgical scrub. A 1 cm skin incision was made on the midline of the ventral cervical region and blunt dissection was used to isolate the left common carotid artery and right external jugular vein.

Model 1: In a method similar to that described by Popovic and Popovic (5), polyethylene tubing (P.E. 50)[2] was used to cannulate both the left common carotid artery and right external jugular vein. The arterial vessel was cannulated and the catheter was advanced 3.5 to 4 cm so that its tip floated downstream in the descending portion of the aortic arch. The venous catheter was inserted 2.5 cm into the superior vena cava or right auricle. Catheters were secured to the vessel with 4-0 surgical silk at three different locations, flushed with heparinized (4 units/ml) isotonic saline, and capped peripherally with a stainless steel plug. The catheters were then routed subcutaneously using 17-gauge stainless steel hypodermic tubing to the dorsal cervical area and exteriorized. Teflon-coated, single stranded, stainless steel wires (34 gauge)[3] were sutured intradermally through a small skin incision on the posterior aspect of all four extremities (Figure 1). The wires were passed subcutaneously to the dorsal cervical region and exteriorized. The animals were fitted with a nylon jacket and spring tether[4], placed in individual wire bottom cages (12" × 12"), and allowed to recover for at least 24 to 48 hours before experimental manipulations were initiated.

Model 2: Rats were prepared for surgery as above and both the left carotid artery and right jugular vein were isolated. A thermocouple probe (0.5 mm O.D.)[5] was inserted into the left carotid artery and advanced 4 mm into the descending thoracic aorta, while P.E. 20 tubing[2] was inserted 2.5 cm into the right auricle or superior vena cava via the right external jugular vein. The probe and P.E. tubing were exteriorized and treated as described above.

Phasic arterial blood pressure was measured with the carotid arterial catheter connected to a strain gauge transducer[6] and transducer amplifier. The arterial catheter was intermittently connected to the transducer during a measurement period. Between measurement periods, the catheter was flushed with heparinized isotonic saline and capped with a stainless steel pin. Six-lead frontal plane electrocardiograms were recorded from the implanted wire electrodes intermittently attached by alligator clamps to an ECG amplifier and chart recorder[7]. Interval and amplitude measurements were made under magnification to the nearest 0.02 millimeter using a vernier caliper[8]. The same individual made the measurements on all ECG tracings. Cardiac output was measured in Model 2 by the thermodilution technique in which 150 to 200 µl of room temperature (20.5 - 22.0°C) normal saline was injected rapidly by a microliter syringe[9] through the venous catheter into the right auricle. Care was taken not to handle the syringe barrel which was held in a clamp. The aortic blood temperature was measured by the thermocouple probe, and the resulting thermodilution curve recorded by a chart recorder. Cardiac output was calculated by a commercial cardiac output computer[10]. The average of two measurements taken 1 minute apart was used as the cardiac output for a given time point. Values were normalized per 100 g body weight. The injectate volume used in the calculation was corrected for the cannula dead space within the animal (8,9).

Hemodynamic measurements: Arterial blood pressures and ECGs were evaluated as part of a toxicological study in a group of 49 F344 rats. Four hundred and thirty-five arterial blood pressure measurements were recorded, and 353 ECG tracings were analyzed before and serially after initiation of experimental treatment. Average values for the control measurements are reported in Tables 1 and 2. Figure 2 demonstrates a representative six-lead ECG tracing. Figure 3 demonstrates a phasic blood pressure tracing with the simultaneously recorded Lead II ECG. Similarly, 197 measurements of cardiac output were accomplished before and serially after experimental treatment in a group of 27 rats. Control or baseline values are reported (Table 1), as well as a representative thermodilution curve (Figure 4). Additionally, 15 control rats were monitored for 7 days. In these rats, mean arterial blood pressures remained near baseline values; whereas,

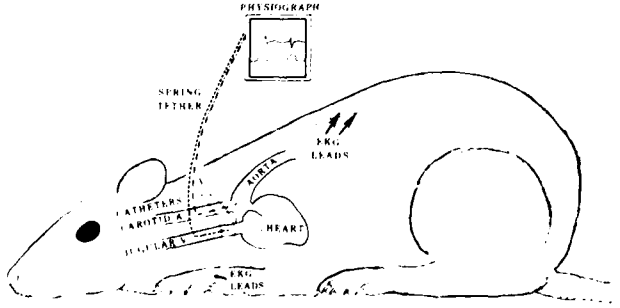

Figure 1 Schematic drawing of Model #1. The rat is instrumented for measurement of arterial blood pressure and simultaneous recording of electrocardiograms. Model #2 differs in having a thermocouple probe instead of a catheter in the carotid artery. Animals are housed individually in square, wire-bottom cages (12 × 12in). Catheters, wires, and/or probes are connected above the animals cage to appropriate physiologic monitoring equipment and chart recorders.

Table 1 Baseline arterial blood pressures and cardiac output in awake F344 rats

	Mean	STD	Min	Max	N
Heart Rate (beats/min)	386	27	330	450	49
Systolic blood pressure (mm Hg)	117	10.4	128	170	49
Diastolic blood pressure (mm Hg)	107	9.5	87	127	49
Mean blood pressure (mm Hg)	121	9.5	102	141	49
Pulse pressure (mm Hg)	40	3.9	31	52	49
Cardiac output (ml·min^{-1}·100g^{-1})	36.4	4.9	30.2	50.4	27

Table 2 Baseline lead II ECG in awake F344 rats

	Mean	STD	Min	Max	N
P wave amplitude (mvolts)	0.107	0.028	0.014	0.15	49
P-R interval (msec)	40.38	0.32	35.00	45.00	49
QRS duration (msec)	12.29	0.29	10.00	15.00	49
R wave amplitude (mvolts)	0.879	0.186	0.550	1.30	49
Q-T interval (msec)	74.59	1.48	57.40	84.80	49
T wave amplitude (mvolts)	0.134	0.032	0.088	0.222	48
Mean QRS axis (degree)	51.0	20.6	15.0	90.0	49

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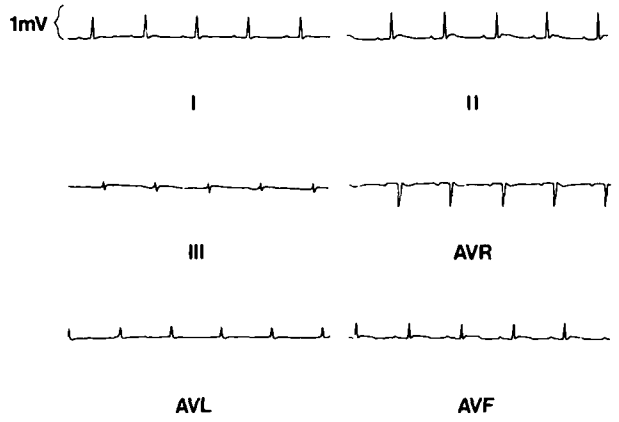

Figure 2 Representative 6-lead frontal plane electrocardiogram from an awake, tethered F344 rat. Paper speed = 100 mm/sec.

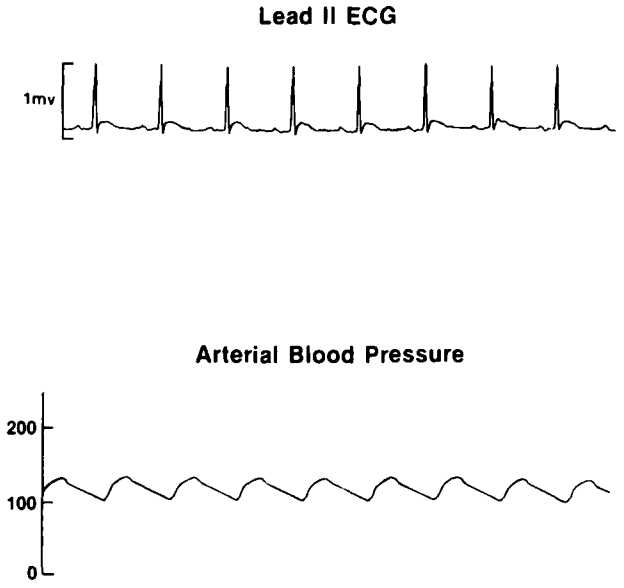

Figure 3 Lead II ECG and simultaneously recorded arterial blood pressure tracing from an awake, tethered F344 rat. Paper speed = 100 mm/sec.

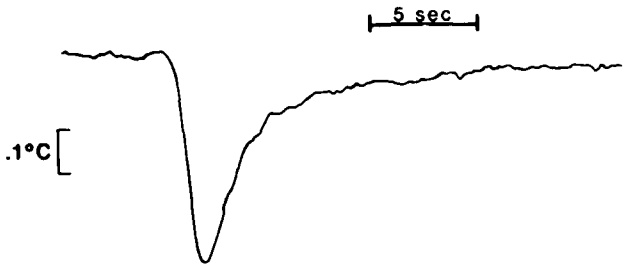

Figure 4 Thermodilution cardiac output curve recorded from a thermocouple probe located in the aortic arch following a bolus injection of 150 µl room temperature isotonic saline into the right auricle.

heart rate tended to decrease (N.S.) by the seventh day (Table 3). Finally, the functional success rate for the model was evaluated over a 7-day period in a control and experimentally treated group. ECGs were determined to be successful if interpretable tracings could be obtained. Arterial blood pressure tracings were considered to be successful if a relatively undampened pulse pressure could be obtained for the accurate determination of mean arterial blood pressure. Arterial blood pressure determinations could be made readily through the 7-day period, while only 64% of the rats had functional ECGs at that time (Table 4). The most common causes for failure were breakage of the implanted wires and the development of 60-cycle interference.

Baseline values in this study agreed closely with values reported for cardiac output, mean arterial blood pressure, and heart rate in other awake models. Literature values for cardiac output are the most varied among the parameters studied and are dependent on the measurement technique, as well as the anesthetic state of the animal. Other investigators utilizing the thermodilution technique in conscious rats have reported values ranging from 28.3 to 51.8 ml·min^{-1}·100g^{-1} (2,8,9,10,11,12). Cardiac output in conscious rats ranges from 20.7 to 45.4 ml·min^{-1}·100g^{-1} when measured by application of the fick principle (1,13,14), dye dilution (15), electromagnetic flow probe (16), and reference sample microsphere method (12,17,18). From our own experience (unpublished observations) and from previously reported values (9,14,15), anesthesia generally causes cardiac output to be depressed as much as 30 to 40%.

Thermodilution cardiac output was utilized because it did not require extensive surgical preparation (i.e., thoracotomy), it easily afforded the ability to make repeated measurements without the necessity for blood withdrawl, and it was inherently safe. Because of the difficulty involved in placing a thermocouple probe in the pulmonary artery of small animals, cardiac output must be measured across the pulmonary circulatory bed by the aortic thermodilution technique. This method tends to overestimate cardiac output due to the loss of thermal indicator in the pulmonary vascular bed (19). Nonetheless, this procedure has been shown to be accurate, reproducible (11,19,20), as well as sensitive enough to detect changes following physiologic or pharmacologic interventions (9,12,21) in small animals like the rat. However, care must be taken to minimize and account for the cannula dead space within the animal. In this study, P.E. 20 tubing was utilized because of its small internal diameter. Utilization of larger internal diameter tubing, (ie. P.E. 50) was found to produce inconsistent results due to the larger dead space.

Phasic arterial blood pressure and heart rate varies with the conscious state of the animal. Anesthetized rats generally have lowered arterial blood pressures and heart rates, but this response is not always clear. Stability and normality of hemodynamic function is also difficult to maintain during prolonged anesthesia. In the present study, individual animals demonstrated stable arterial blood pressures and heart rates only when resting quietly in the cage. Grooming, drinking and other natural behaviors, however, resulted in spontaneous excitation of

Table 3 Arterial blood pressures and heart rates in awake F344 rats over a 7-day period

	Day 0	Day 1	Day 2	Day 3	Day 7
	n = 15	n = 15	n = 15	n = 15	n = 14
Heart Rate (beats min $^{-1}$)	391 ± 20[a]	383 ± 39	376 ± 32	372 ± 35	373 ± 32
Systolic blood Pressure (mm Hg)	148 ± 11	141 ± 14	149 ± 15	143 ± 13	148 ± 13
Diastolic Blood Pressure (mm Hg)	107 ± 10	102 ± 13	108 ± 13	102 ± 13	109 ± 12
Mean Blood Pressure (mm Hg)	121 ± 10	115 ± 13	121 ± 13	116 ± 12	122 ± 13
Pulse Pressure (mm Hg)	41 ± 4	39 ± 3	41 ± 4	41 ± 4	39 ± 4

[a]Mean ± standard deviation

Table 4 Success rate over time of ECG and arterial blood pressure model

	Day 0	Day 1	Day 2	Day 3	Day 7
ECG	25/25[a] (100%)	24/25 (96%)	23/25 (92%)	21/25 (84%)	16/25 (64%)
Arterial Blood Pressure	25/25 (100%)	25/25 (100%)	25/25 (100%)	25/25 (100%)	24/25 (96%)

[a]Values represent number of rats that had functional electrode wires and catheters.

heart rate and arterial pressure as previously reported (22). Therefore, measurements were taken when the animals were calm to reflect basal levels.

There are many reports in the literature evaluating electrocardiographic alterations in the rat, but few have utilized an awake, relatively undisturbed model. In our hands, tracings adequate for measuring intervals and amplitudes were achieved consistently. The measured amplitudes and intervals agreed closely with those previously published for the lightly anesthetized rat (23,24). This capability allowed monitoring of rate and rhythm, as well as the ability to observe subtle changes in the ECG pattern.

The uniqueness of the model lies in its ability to measure arterial blood pressure, as well as ECG, simultaneously. This, coupled with cardiac output, provided a much better overall cardiovascular profile than could be attained from arterial blood pressure and heart rate alone. However, the procedure utilized in this study did not permit the measurement of cardiac output and arterial blood pressure in the same individual. We have attempted femoral arterial cannulation for blood pressure measurement in rats also instrumented for the measurement of cardiac output, but have observed an unacceptable number of ischemic injuries in the rear leg distal to the catheter placement site. Although femoral arterial cannulation for chronically catheterized rats are reported commonly in the literature, few investigators have reported this associated injury.

The techniques described have been utilized successfully in this laboratory to evaluate cardiac output, arterial blood pressure and ECG in a large group of F344 rats without the uncertainty of anesthetic/drug interactions. Individual animals have been followed for up to 1 week during which electrocardiographic and hemodynamic measurements were successfully obtained. Rats seem readily suited to the tether system described, as they are able to move freely and rarely disturb the jacket or tether. There appears to be minimal discomfort after instrumentation, because most animals return to food and water within 24 to 48 hours. The result is a relatively unstressed awake model, free from the influence of anesthesia and physical restraint.

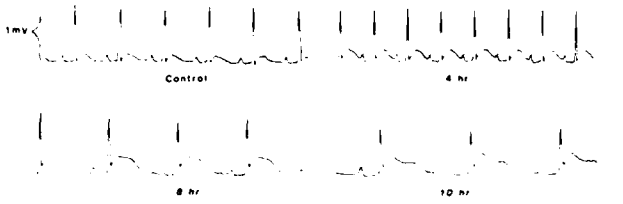

Figure 5 Lead II electrocardiogram at control, 4, 8 and 10 hours following iv administration of T-2 mycotoxin (1mg/kg). Note biphasic heart rate response consisting of an increase at 4 hours followed by a decrease in heart rate at 8 and 10 hours. Other changes include J-point elevation, P-R interval prolongation, and T-wave changes consistent with myocardial ischemia.

Cardiovascular toxicology is one example of a discipline that can utilize this model. An example of toxin-induced electrocardiographic alterations is illustrated in Figure 5. The tracings show that changes in ECG intervals and amplitudes can be easily assessed and quantified in this model. In this case, 1mg/kg T-2 mycotoxin resulted in a biphasic heart rate response consisting of an increase at 4 hours followed by a decrease in heart rate at 8 and 10 hours. Other changes that are obvious in this rat include J-point elevation, P-R interval prolongation and T-wave changes.

Acknowledgements

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", as promulgated by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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Footnotes

[1]Charles River Breeding Laboratory, Kingston, NY.
[2]Clay Adams, Parsippany, NJ.
[3]Jersey Strand and Cable Inc, Washington, NJ.
[4]Alice King and Chatham, Los Angeles, CA.
[5]Bailey Instruments Inc., Saddlebrook, NJ.
[6]Statham P23iD, Gould Inc., Oxnard, CA.
[7]Gould 2600, Gould Inc., Oxnard, CA.
[8]Vernier Caliper, Mitutoyo, Tokyo, Japan.
[9]Hamilton microliter #750, Hamilton Co., Reno, NV.
[10]Cardiotherm 500 AC-R, Columbus Instruments, Columbus, OH.